Morphological Characterization of the *Littorina scutulata* Species Complex

BY

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(2 Text figures)

INTRODUCTION

Gould (1849) described three species of *Littorina* (*L. lepida, L. plena* and *L. scutulata*) from the northeastern Pacific coast that have subsequently been considered as morphological variants of *L. scutulata*. On the basis of reproductive characteristics, however, Murray (1979) demonstrated that this taxon was a mixture of two species but was unable to identify morphological differences between the two species. The confounding of the two species has remained a problem, due to the variance and degree of overlap in univariate morphometric characteristics. In this paper I have employed the multivariate statistical techniques of discriminant analysis to identify morphological differences between the reproductive types of Murray (*op. cit.*) and principal component analysis to describe size and shape variation between the two species. It was also possible using discriminant analysis to classify existing type material to one of the two species and to identify synonymies in Gould's (1849) material.

This study would not have been possible without the loan of type material by Dr. J. Rosewater, U.S. National Museum of Natural History, Washington, D.C. and Dr. K. J. Boss, Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts for which I am grateful.

METHODS

Statistical analyses were based on whorl number, shell length, whorl height, width perpendicular to the columellar axis, maximal width, shell depth and apical angle of preserved females of known spawning history from Murray's (1979) study. Linear measurements were taken with a pair of vernier calipers to the nearest 0.01 mm (see Figure 1). Apical angle (in radians) was derived as the arc tangent of the shell length: maximal width ratio. All measurements were made with the aperture oriented upwards. The discriminant and principal component analyses were conducted using the Biomedical Computer Programs — P series library version BMDP-77 developed by the Health Sciences Computing Facility, UCLA, Los Angeles, California. The discriminant analysis (BMDP-7M, program revised November 1979) employed a stepwise variable entry procedure followed by stepwise variable removal to maximise the generation of alternative models for highlighting morphological differences (see Draper & Smith, 1966). The classification of individuals to species was based on a jackknife estimation procedure in order to minimise bias. Principal components were extracted from the covariance matrix of log transformed measurements of shell length, whorl height, width perpendicular to the columellar axis, maximal width and shell depth after the suggestion of Jolicoeur (1963) using BMDP-4M (program revised November 1979). Whorl number could not be included because it is measured in units that give it a minimal contribution to the principal components (Jolicoeur, *op. cit.*).

Morphological Differences Associated with Reproductive Types within the *Littorina scutulata* Complex

The earlier study of Murray (1979) established that two species were being confounded as *Littorina scutulata*. However, without knowledge of the age of individuals the differences in the means of the morphometrics could not be ascribed to species differences. In addition, the overlap in the distributions of each character meant that univariate traits could not be used to tell the species apart reliably.

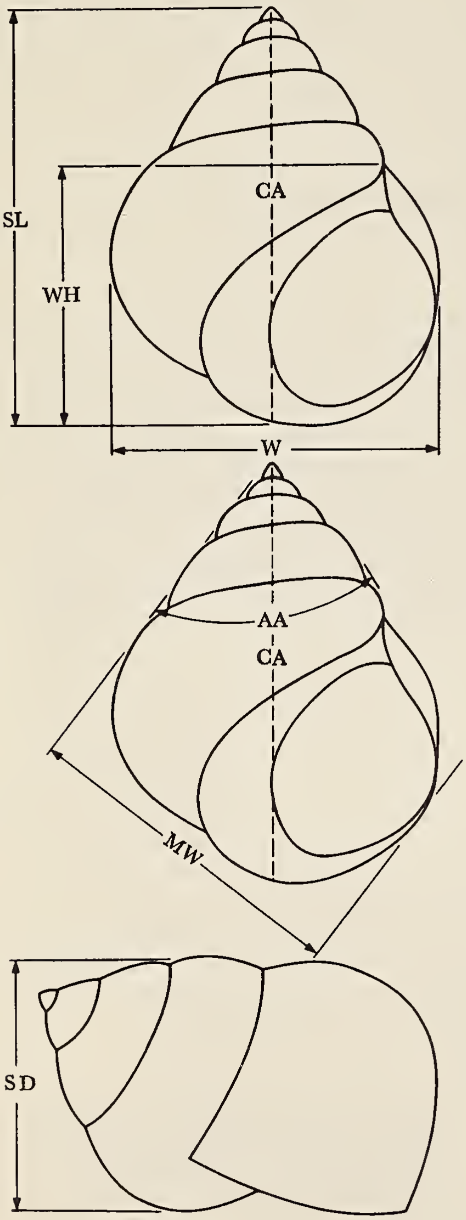

Figure 1

Morphological measurements made on each shell.
AA – apical angle · CA – columellar axis
MW – maximal width SD – shell depth SL – shell length
W – width WH – whorl height

Discriminant analysis is ideally suited to the resolution of such problems. It is an extremely robust procedure that allows one to combine morphometrics (*e.g.*, lengths, weights, angles, etc.) with morphological traits (*e.g.*, number of whorls, presence or absence of a trait, etc.) to construct divisive criteria amongst groups known to be distinct based on some independent criterion. The divisive criteria are linear functions of morphological characters that yield the widest separation of groups. In the present application we know the two species differ in their reproductive characteristics and hypothesize the existence of an unknown set of concomitant morphological differences. We do not know *a priori* what this set will be, hence we employ a stepwise variable entry procedure that will produce a maximum number of alternative discriminant models. We subsequently choose the set of variables that yields discriminant functions with the highest proportion of correct classifications of reproductive types and of type material. If the probability of correctly classifying individuals is sufficiently better than by chance, we accept our hypothesis that there exist morphological differences between the reproductive types. We can then employ these functions to identify individuals of unknown reproductive history.

In this study the best discriminant model for separating the two species is the following pair of four variable functions: $Z_1 = 15.71X_1 - 12.21X_2 + 20.58X_3 + 4.75X_4 - 51.48$; $Z_2 = 13.34X_1 - 20.39X_2 + 33.06X_3 + 12.11 X_4 - 70.57$ where: X_1 = whorl number, X_2 = shell length, X_3 = whorl height, and X_4 = shell depth. The classificatory power of these functions is indicated in Table 1. With these functions 95.6% of the specimens known to be *Littorina scutulata* were correctly classified and 96.0% of the specimens known to be different from *L. scutulata* were also correctly classified. Type material of *L. scutulata,* with the exception of 3 of 5 idiotypes from San Francisco (MCZ 169360), was also correctly classified. It seems probable that this sample and that of USNM 796179 are mixtures of the two species, since the probability of misclassification is small (4%) and the collecting locality is an area where both species commonly co-occur (MURRAY, 1979).

Type material of *Littorina plena* and *L. lepida* was consistently classified as different from *L. scutulata.* Since the type specimens of *L. plena* and *L. lepida* cannot be distinguished from each other, they should be regarded as conspecific. As first reviser I propose the reinstatement of *Littorina plena* (to be described later in this paper) for the other member of the *L. scutulata* complex. *Littorina lepida* should be regarded as a synonym of *L. plena.* I regard *L. plena* as a preferable name, since

Table 1

Classification of material examined based on discriminant analysis of *Littorina scutulata* reproductive types I and II of MURRAY (1979).

Group	% correctly classified	Number of cases classified as *Littorina scutulata*	Number of cases classified as Not *Littorina scutulata*	Comments
Littorina scutulata	95.6	43	2	Murray's (1979) Type II ♀ ♀
Not *Littorina scutulata*	96.0	2	48	Murray's (1970) Type I ♀ ♀
MCZ 169222	100.0	0	4	*Littorina lepida* syntypes, Puget Sound
MCZ 169289	100.0	0	2	*Littorina plena* idiotypes, San Francisco
MCZ 169360	mixture	2[1]	3	*Littorina scutulata* idiotypes, San Francisco
USNM 5635	100.0	0	1	*Littorina plena* type, San Francisco
USNM 5637	100.0	1	0	*Littorina scutulata* figured type, Puget Sound
USNM 5640	100.0	1	0	*Littorina scutulata* holotype, Puget Sound
USNM 612308	100.0	1	0	*Littorina scutulata* paratype, Puget Sound
USNM 677095	100.0	0	1	*Littorina lepida* paratype, Puget Sound
USNM 677096	100.0	0	2	*Littorina plena* paratype, San Francisco
USNM 796179	mixture	4[2]	6	*Littorina scutulata* from San Francisco

[1]shell lengths = 10.33, 8.18 mm

[2]shell lengths = 5.95, 8.55, 8.60, 8.95 mm

the description of GOULD (1849: 84) more closely describes the specimens I have observed than does his description of *L. lepida* (GOULD, 1849: 83) thereby overriding page precedence of the Law of Priority (article 24 of the ICZN).

The discriminant functions Z_1 and Z_2 have a wider utility outside the scope of this paper. Since the two species are easily confused, these functions can be used in future studies to improve the accuracy of identification using four easily measured shell characters. The following example demonstrates how the functions Z_1 and Z_2 are used. We collect a shell and record its number of whorls (X_1) as 5.0, shell length (X_2) as 8.10 mm, whorl height (X_3) as 4.59 mm, and shell depth (X_4) as 4.69 mm. We then calculate values for Z_1 and Z_2 given these data. We classify our specimen according to the following inequalities: if $Z_1 < Z_2$ the specimen is *Littorina scutulata,* if $Z_1 > Z_2$ the specimen is *L. plena.* In our example we calculate values of $Z_1 = 44.91$ and $Z_2 = 39.51$, therefore, since $Z_1 > Z_2$ we predict (with greater than 95% certainty) that the specimen is *L. plena.* This technique is extremely powerful and has been used by MURRAY (1980) to demonstrate morphological differences within a species due to tidal height and parasitism. The reader should bear in mind that these functions do not take into account morphological variation that might exist between sexes, tidal level, or differences that result from other environmental sources. This cautionary note does not denigrate the utility of these functions but they should be used with caution. They are included because we expect that differences between species are greater than differences within a species.

Morphological Variation in Size and Shape in *Littorina scutulata* and *Littorina plena*

Thus far we have assumed different shell morphologies between species and used these differences without characterizing them. We can now proceed to develop multivariate measures of size and shape for *Littorina scutulata* and *L. plena,* using principal component analysis. The advantage of this technique is that it allows one to develop the best single descriptions of mutually independent trends in size and shape variability. Best is used in the sense that each trend maximally explains the observed variation in morphology with respect to that trend. For example, each description of size variation (= first principal component) maximally describes the variability that can be attributed to variation in shell morphology associated with differences in size among specimens. The remaining information about morphology is explained by variation in shape that is independent of size and derived from the residual variance of the first principal component. This process is continued until the information gained at each extraction tails off.

Variation in size and shape together explain more than 98% of the total variation in shell morphology in *Littorina scutulata* and in *L. plena,* so additional principal components were not extracted. Most of the variation in morphology is associated with size related variation, as can be seen from Table 2 (97.3% for *L. scutulata* and 97.5% for *L. plena*). Variation in morphology attributable to shape is small (1.3% for *L. scutulata* and 1.9% for *L. plena*). Although shape is a small component of morphology, it is shape variation that exhibits the greatest difference between species and probably accounts for our ability to separate *L. scutulata* and *L. plena* morphologically. This becomes readily apparent when we calculate the angle between each trend in variation. It should be borne in mind that each principal component is a vector (whose elements are the coefficients in Table 2) extending through a cluster of points that represents the shell geometry of each species. Since it is a vector, we can calculate the angle between principal components as a measure of the difference between species. If we take $U_1 = u_{11}, u_{12}, u_{13}, u_{14}, u_{15}$ as the vector of coefficients associated with size variation in *L. scutulata* and $V_1 = v_{11}, v_{12}, v_{13}, v_{14}, v_{15}$ as the vector of coefficients associated with size variation in *L. plena* then the angle between U_1 and V_1 is given by: $\cos\theta = u_{11}v_{11} + u_{12}v_{12} + \ldots + u_{15}v_{15}$ (JOLICOEUR, 1963). The more similar the trends the smaller the angle θ. Using the coefficients in Table 2 we calculate an angle of 3.1° between the trends in size variation in *L. scutulata* and *L. plena.* Shape variation between the species is more widely divergent and is separated by an angle of 9.9°. Shape differs more than size between species.

Graphically, we can view each trend as a morphological continuum with a greater separation of the species along the shape continuum than along the size continuum. This separation of species along the shape continuum is evident in Figure 2. The ellipses have been drawn in to delimit the groups and do not represent confidence ellipses. This figure does serve to dramatize the different morphologies for *Littorina scutulata* and *L. plena.* We can now describe the characteristics of each and highlight the differences between species.

Littorina scutulata Gould, 1849

holotype: USNM 5640, collected Puget Sound, shell length = 12.16 mm

paratype: USNM 612308, collected Puget Sound, shell length = 8.79 mm

Littorina scutulata are small obconic brown snails of the mid-littoral zone. Adult snails typically have 5 whorls and can be expected to range in size from 11.3 mm to 11.9 mm (mean shell length = 11.61 mm). Infrequently (15.9% of the snails observed) the base of the body whorl will be marked by an indistinct ivory colored band which may also be visible as a stripe inside the aperture. The apical angle is typically 30.4°. Morphological characters are summarized in Table 3. The reproductive features of this species definitively identify it (MURRAY, 1979). Males possess a penis with a conspicuous sperm groove running dorsally to a sub-terminal bulge. Female *L. scutulata* produce characteristic pelagic egg capsules shaped like inverted saucers 0.7 to 1.0 mm in diameter and containing 1 to 14 eggs 105 μm in diameter.

Table 2

Coefficients of size and shape functions for *Littorina scutulata* and *Littorina plena.*

	Littorina scutulata		*Littorina plena*	
	size variation (1st P.C.)	shape variation (2nd P.C.)	size variation (1st P.C.)	shape variation (2nd P.C.)
Log shell length	0.4907	0.2659	0.5012	0.2152
Log whorl height	0.4083	−0.2926	0.3740	−0.4460
Log width	0.4399	−0.4361	0.4216	−0.3823
Log maximal width	0.4267	−0.3640	0.4187	−0.3317
Log shell depth	0.4657	0.7202	0.5062	0.7080
Variance × 10^4	229.86	3.11	414.11	7.98
% total variance	97.30	1.32	97.53	1.88

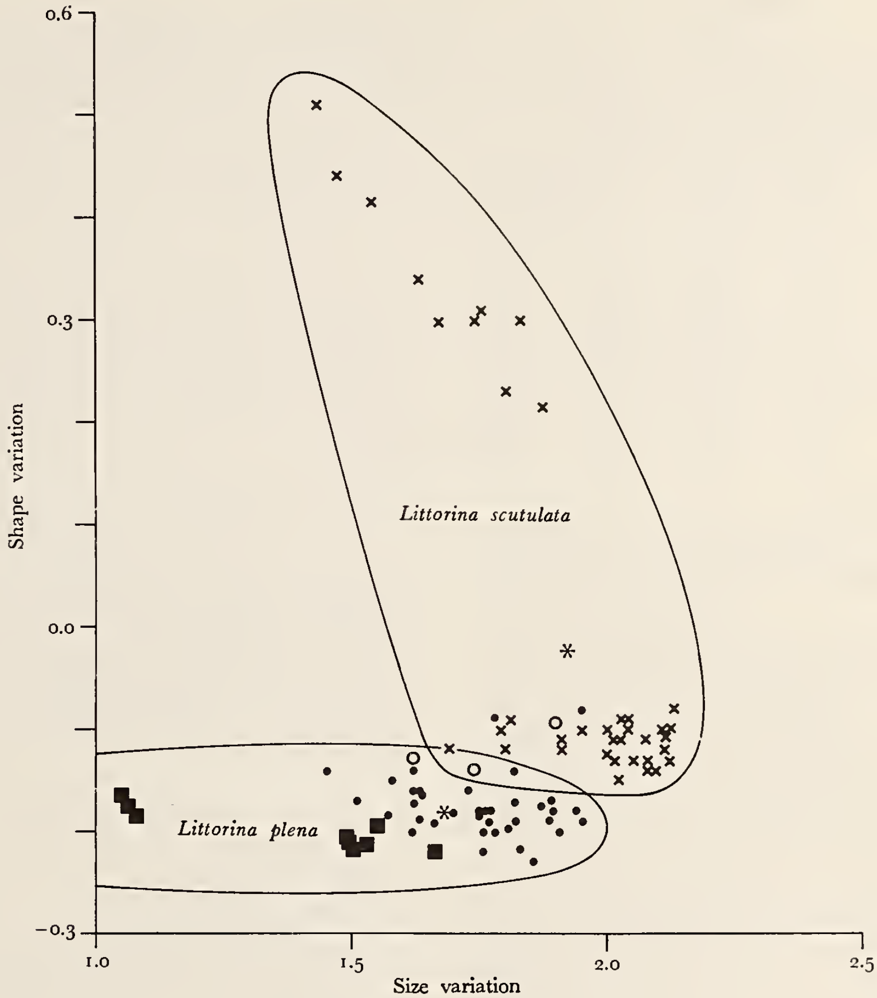

Figure 2

Scatterplot of size versus shape variation in *Littorina scutulata* and *Littorina plena*

Asterisks (*) represent the mean of each group; × represents *Littorina scutulata* from Dillon Beach, California; open circles (○) *L. scutulata* from Rockport, California; closed circles (●) *L. plena* from Newport, Oregon; closed squares (■) *L. plena* from Dillon Beach, California. Values of size and shape variation were calculated from the coefficients in Table 2

Littorina plena Gould, 1849

(synonym: *Littorina lepida* Gould, 1849)

holotype: USNM 5635, collected San Francisco, shell length = 8.52 mm
paratypes: USNM 677096, collected San Francisco, shell lengths = 6.22, 8.20 mm

The morphological characteristics of *Littorina plena* are summarized in Table 3. Although this species is morphologically similar to *L. scutulata,* adults are generally smaller (8.4 mm < shell length < 8.9 mm as opposed to 11.3 mm < shell length < 11.9 mm). *Littorina plena* as noted by Gould (1849) also tend to be more ovate, having

Table 3

Summary of the morphological characteristics of *Littorina scutulata* and *Littorina plena.*

	Littorina scutulata		*Littorina plena*	
	mean (± 1 S.E.)		mean (± 1 S.E.)	
Whorl number	5.09	(0.08)	5.40	(0.10)
Shell length (mm)	11.61	(0.28)	8.60	(0.25)
Whorl height (mm)	7.00	(0.14)	4.87	(0.11)
Width (mm)	7.81	(0.17)	5.44	(0.13)
Maximal width (mm)	6.84	(0.52)	6.35	(0.16)
Shell depth (mm)	6.37	(0.15)	4.53	(0.14)
Apical angle (radians)	0.53	(0.04)	0.64	(0.004)
Size (1st P.C.)	1.92	(0.03)	1.68	(0.03)
Shape (2nd P.C.)	−0.02	(0.03)	−0.18	(0.004)
Frequency of band on the base of the body whorl	15.9%		84.3%	
n	45		50	

an apical angle of 36.7° as opposed to 30.4° in *L. scutulata.* The ivory band present but indistinct in 15.9% of *L. scutulata* is more prominent in *L. plena* and occurs on 84.3% of the shells studied. The coloration of *L. plena* shells is also more variable than *L. scutulata* and ranges from dark brown to brown tessellated with grey. As in *L. scutulata,* the reproductive features described by MURRAY (1979) definitively characterize this species. Male *L. plena* have a penis in which the sperm groove runs laterally to the tip instead of dorsally as in *L. scutulata.* The penis of *L. plena* also bears a prominent papilla on the dorso-lateral surface proximal to the curvature of the penis. This papilla is absent in *L. scutulata.* Female *L. plena* also differ from *L. scutulata* in the characteristics of their spawn. Female *L. plena* produce pelagic egg capsules that resemble automobile wheels slightly greater than 1 mm in diameter and contain from 4 to 41 eggs 95.7 μm in diameter.

CONCLUSIONS

Discriminant analysis verified the hypothesis that in addition to the reproductive differences in *Littorina scutulata* species complex reported by MURRAY (1979) there exist associated morphological differences. Using principal components it was possible to demonstrate that most of the difference in morphology between species could be attributed to a separation along a multivariate shape continuum. However, the primary purpose of this study is to provide a way of minimizing the confounding of *Littorina scutulata* and *L. plena* in future studies. The ease with which these species can be distinguished is ultimately dependent upon the level of accuracy a researcher is content with. If a researcher is content with an approximate 16% chance of error, then *L. plena* can be distinguished by the presence of an ivory band on the base of the body whorl and its generally smaller size. This error rate, however, can be reduced to approximately 4% by counting the number of body whorls, measuring shell length, whorl height and shell depth and employing the discriminant functions derived in the text. If a 4% error rate is unacceptable, the two species can be distinguished with absolute certainty only by recourse to the reproductive characteristics described by MURRAY (1979). In addition to the low error rate, the discriminant functions have the additional advantages that they do not require snails in breeding condition; they work equally well on empty shells as on live material; the necessary measurements are easily made and usually of considerable interest in a study; and the calculations necessary to determine species identity are readily done on a hand calculator.

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